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THE EFFECT OF β-GALACTOSIDE ACCUMULATION ON THE UPTAKE OF PHOSPHATE INTO CELLS AND CELL NUCLEOTIDES OF *ESCHERICHIA COLI*

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SUMMARY

The effect of the accumulation of β-galactosides on the uptake of P_i into cells and cell nucleotides was examined in ML strains of *Escherichia coli*. Nonmetabolizable sulfur analogs of lactose, which are accumulated only in the presence of the product of the y gene of the *Lac* operon, inhibited the uptake of P_i into whole cells and into cell nucleotides. This inhibition was most pronounced in starved cells, those with a low rate of ATP production. When the cell membrane was disrupted by sonication or detergents, the inhibition was lost. No significant inhibition was seen in y^- strains or in inducible y^+ strains which were not induced. Hence, inhibition of the uptake of phosphate into nucleotides is dependent on the presence of the product of the y gene and a β-galactoside.

A technique using $^{32}P_i$ and $^{33}P_i$ was developed for simultaneously measuring the turnover and level of nucleotides. β-Galactosides inhibited ATP synthesis in aerobic cells, but stimulated ATP synthesis in anaerobic cells, indicating that an intermediate of oxidative phosphorylation was the source of energy for β-galactoside accumulation.

INTRODUCTION

The accumulation of β-galactosides by *Escherichia coli* is mediated by the M protein[1], and requires the expenditure of metabolic energy[2]. The site of coupling of metabolic energy to the M protein has not been definitively established, and is the subject of this paper.

A number of studies have reported on the effects of inhibitors of energy metabolism on the accumulation of β-galactosides. In aerobic cells, inhibitors of oxidative phosphorylation and electron transport inhibit the accumulation of β-galactosides[2–5], suggesting that oxidative phosphorylation can provide the energy for β-galactoside accumulation. Inhibitors of oxidative phosphorylation also inhibit β-galactoside accumulation in anaerobic cells[6]. Since ATP levels were not affected[6], one could conclude that in anaerobic cells, ATP, produced by glycolysis, generates

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; TDG, thiodigalactoside; IPTG, isopropylthiogalactoside; PTG, phenylthiogalactoside; TMG, methylthiogalactoside.

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a high energy intermediate, which is sensitive to inhibitors of oxidative phosphorylation and is the source of energy for β-galactoside accumulation.

This paper presents data which show more directly that an intermediate of oxidative phosphorylation is the source of energy for β-galactoside accumulation in *E. coli*. The approach has been to measure the effect of β-galactoside accumulation on the level and turnover of nucleotides in aerobic and anaerobic cells.

MATERIALS AND METHODS

Materials

Chemicals and enzymes were purchased from the following sources: thiodigalactoside (TDG) and isopropylthiogalactoside (IPTG) from Mann Research Laboratories; phenylthiogalactoside (PTG) and methylthiogalactoside (TMG) from Sigma Chemical Company; nucleotides from P-L Biochemicals; Darco G 60 charcoal from Fisher Scientific Company; acid-washed Norit A charcoal from Pfanstiehl Laboratories; KCN from Mallinckrodt Chemical Works; carbonylcyanide *m*-chlorophenylhydrazone (CCCP) from DuPont Chemical Company; 2-octanol and 2-mercaptoethanol from Eastman Organic Chemicals; polyethyleneimine–cellulose thin-layer chromatography plates from Brinkman Instruments; lysozyme and DNAase from Worthington Biochemical Corporation; [^{3}H]ATP from New England Nuclear Corporation; $^{32}P_i$ and $^{33}P_i$, as carrier-free isotopes in 0.02 M HCl, from New England Nuclear Corporation. The isotopes were diluted 10-fold with water, mixed with 50 mg of acid-washed Norit A charcoal, and filtered. ^{3}H-labeled TDG was prepared by New England Nuclear Corporation and purified by cellulose chromatography.

Growth and harvesting of cells

Cultures of *E. coli* ML 308 ($i^-z^+y^+a^+$), ML 30 ($i^+z^+y^+a^+$) and ML 35 ($i^-z^+y^-a^-$), originally isolated at the Institute Pasteur, were the gift of Dr Arthur Koch. Aerobic cells were grown at 37 °C, with shaking in medium 63 (ref. 7) or in a growth medium of the following composition: 3 mM $MgSO_4$, 5 mM $(NH_4)_2SO_4$, 20 mM KCl, 0.01 mM $FeSO_4$, 1 mM P_i as the potassium salt and 100 mM Tris–HCl, pH 7.0 (hereafter as tris growth medium). Potassium succinate was added as a carbon source to a final concentration of 15 mM. The cells were chilled on ice in mid log phase (0.15 mg of protein/ml), harvested by centrifugation, washed twice with a buffer of the following composition: 1 mM $MgSO_4$, 10 mM KCl and 15 mM Tris–HCl, pH 7.8 (hereafter as buffer A), and resuspended in the same buffer.

Anaerobic cells were prepared by growing cells aerobically at 37 °C, with shaking, in tris growth medium with 1% glycerol as a carbon source. The cells were harvested by centrifugation, washed twice, and resuspended in a buffer of the following composition: 5 mM $(NH_4)_2SO_4$, 64 mM KCl, 3 mM $MgSO_4$ and 100 mM Tris–HCl, pH 7.8 (hereafter as buffer B). The cells were made dependent on glycolysis for energy by a 15-min incubation at 20 °C in the presence of buffer B plus 1 mM KCN and 10 mM glucose.

Accumulation of TDG

Aerobic and anaerobic cells were tested for their ability to accumulate ^{3}H-labeled TDG under the various assay conditions used to measure $^{32}P_i$ uptake. The

cells were incubated with ^{3}H-labeled TDG, collected on a membrane filter, and washed with 1 ml of the assay buffer. The cells were dried and counted in 10 ml of Buhler's scintillation fluid[8] with a Packard Tricarb liquid scintillation spectrometer.

Preparation of cell fractions

Spheroplasts were prepared from washed cells of ML 308 (3 mg protein/ml) by incubating them for 20 min at 30 °C in the presence of 5 mM EDTA, 1 mM 2-mercaptoethanol, 0.3 M KCl, 0.1 mg/ml lysozyme and 50 mM Tris–HCl, pH 7.0. The osmotically sensitive spheroplasts were washed and resuspended in a buffer containing 0.3 M KCl, 1 mM 2-mercaptoethanol, 1 mM $MgSO_4$ and 15 mM Tris–HCl, pH 7.8. Membrane vesicles were prepared from the lysozyme spheroplasts[9], and resuspended in the same buffer used to resuspended the spheroplasts. Cells and spheroplast membranes were disrupted with a Branson sonifier. Unbroken cells were removed by centrifuging 15 min at $3000 \times g$ and carefully decanting the supernatant. The $3000 \times g$ supernatant was centrifuged 30 min at $40000 \times g$ to sediment heavy particles, leaving light particles and the soluble fraction in the $40000 \times g$ supernatant. Protein was determined by the method of Lowry *et al.*[10], with appropriate corrections for Tris and 2-mercaptoethanol.

Measurement of P_i uptake into cells and nucleotides of cells and cell fractions

Cells were grown aerobically in medium 63 and succinate. They were washed twice and resuspended in buffer A. Spheroplasts, spheroplast membranes, sonicates and particulate fractions were prepared from these cells and resuspended in buffer A containing 1 mM 2-mercaptoethanol and 0.3 M KCl. After a 15-min incubation at 20 °C, $^{32}P_i$ was added, with or without a nonmetabolizable β-galactoside, to give a final concentration of 0.01 mM $^{32}P_i$, and the incubation was continued for 15 min at 20 °C. When it was desired to measure the uptake of $^{32}P_i$ into cells, aliquots of the incubating cells were collected on a membrane filter and washed with 1 ml of buffer A. The cells were dried and counted in 10 ml of Buhler's scintillation fluid.

When measuring the uptake of $^{32}P_i$ into the nucleotides of cells and cell fractions two different methods were used. In the first method the cells were diluted into 3 volumes of ice-cold 5% $HClO_4$, and centrifuged. The supernatant was removed and treated with 25 mg of acid-washed Norit A charcoal. After thorough mixing, the charcoal was filtered on a membrane filter and washed with 5 ml of ice-cold 5% $HClO_4$, 5 ml of water and 1 ml of 0.5% casein. The charcoal was allowed to dry at room temperature and was counted directly with a gas-flow Geiger counter. In some experiments the material which was insoluble in $HClO_4$ was not removed by centrifugation, in which case it was retained with the charcoal on the membrane filter. The ^{32}P in these samples was about 6 times that obtained when just the material which was soluble in $HClO_4$ was assayed. The inhibition due to β-galactosides was the same for both measurements. In the second method for measuring $^{32}P_i$ incorporation into the soluble nucleotides, cells were collected on a membrane filter, washed with 1 ml of Buffer A, and then with 2 ml of ice-cold 5% $HClO_4$ to extract the nucleotides. Norit A charcoal was added to the $HClO_4$ extract, and the rest of the procedure was the same as the first method. The two methods gave the same result. When comparing the uptake of $^{32}P_i$ into cells with its uptake into nucleotides simultaneous aliquots were taken from the same culture for the two determinations.

Turnover and level of individual nucleotides

Cells of strain ML 308 were grown in Tris growth medium containing glycerol or succinate and 1 mM $^{32}P_i$ (1–5 μCi/ml growth medium). They were harvested, washed, and incubated 15 min at 20 °C in buffer B. $^{33}P_i$ (1–5 μCi/ml incubation medium) was added with or without TDG to give a final concentration of 50 μM $^{33}P_i$ and 0.0 or 0.5 mM TDG. At various times samples were simultaneously removed for membrane filtration and for treatment with an equal volume of ice-cold 10% $HClO_4$. The filtered cells were washed immediately with 1 ml of buffer B, dried, placed in 10 ml of Buhler's scintillation fluid, and counted for ^{33}P and ^{32}P with a Packard Tricarb liquid scintillation spectrometer. The material which was insoluble in $HClO_4$ was removed by centrifugation. The supernatants were mixed with 100 mg of Darco G 60 charcoal, which had been treated with 2-octanol[11], and allowed to stand on ice for 15 min. The charcoal was filtered on a millipore filter, washed with 20 ml of water, and the nucleotides were eluted with about 20 ml of ammonia–ethanol–water (1:50:49, v/v/v). About 85% of the nucleotides were eluted. The eluents were dried and taken up in 50 μl of water for chromatography on polyethyleneimine–cellulose thin-layer chromatography plates[12]. Unlabeled nucleotide standards were added to the $HClO_4$ extracts before the addition of charcoal in order to characterize the spots on the completed chromatograms. The nucleotides were visualized with ultraviolet light and autoradiography. Separate aliquots were chromatographed by a modified procedure to separate deoxy- from ribonucleoside triphosphates[13]. The nucleotides were removed from the plates for counting by cutting them out and boiling them for 10 min in 2 ml of 1 M HCl. 1 ml of the HCl extract was added to 10 ml of Patterson–Greene scintillation fluid[14], and the radioactivity of ^{32}P and ^{33}P isotopes was determined with minimal overlap. An overall recovery of 80% was obtained when [^{3}H]ATP was carried through the entire procedure. About 95% of the [^{3}H]ATP could be accounted for by counting the charcoal and applying a correction for the [^{3}H]ATP not eluted from the charcoal.

The endogenous pool of P_i was determined by the same procedure as above, except unlabeled P_i was used in place of $^{33}P_i$. Cells treated with $HClO_4$ were mixed with 25 mg of Norit A charcoal and P_i to give a final concentration of 1.5 mM P_i. The charcoal and insoluble material were removed by centrifugation, and the $^{32}P_i$ in the $HClO_4$ supernatants was complexed with molybdate and extracted into benzene[15]. The benzene was evaporated, the residue was dissolved in 1 ml of 1 M HCl, 10 ml of Patterson–Greene scintillation fluid was added, and the ^{32}P activity was determined.

In calculating the nmoles of nucleotide phosphorus/mg of protein, $^{33}P/^{32}P$, and $^{33}P_i$ incorporated into nucleotides/mg cell protein, corrections were made for changes in the endogenous pool of P_i, nucleotides not eluted from the charcoal, and the overlap of the ^{33}P and ^{32}P (less than 5% for each isotope).

RESULTS

The effect of β-galactosides on the uptake of P_i into aerobic cells and nucleotides of cells and cell fractions

Under all of the assay conditions used, ^{3}H-labeled TDG was accumulated to a level of 20–50 times the level necessary to equilibrate the cells with the external

TABLE I

Inhibition by β-galactosides of the uptake of exogenous P_i into cells and cell nucleotides. Assays were started with the addition of $^{32}P_i$ to a final concentration of 0.01 mM. β-Galactosides were added with the $^{32}P_i$ to give the concentrations shown. Uptake into the nucleotides was determined by filtering the cells on a millipore filter, extracting the nucleotides with $HClO_4$, and adsorbing them to charcoal (see text for details). The values shown are the percent inhibition of P_i uptake into nucleotides due to the presence of the indicated β-galactoside, and are the averages of duplicate determinations. Individual values ranged between ±10% of the mean.

β-Galactoside	*Strain*	*Per cent inhibition*	
		Into cells	*Into cell nucleotides*
5 mM TDG	ML 308	24	49
5 mM IPTG	ML 308	—	71
5 mM TMG	ML 308	—	53
5 mM PTG	ML 308	—	51
5 mM TDG	ML 308	—	68
1 mM TDG	ML 308	—	54
0.5 mM TDG	ML 308	—	53
0.1 mM TDG	ML 308	—	47
0.01 mM TDG	ML 308	—	44
5 mM PTG	ML 35	—	−5
0.5 mM TDG	ML 30, uninduced	—	11
0.5 mM TDG	ML 30, induced*	—	59

* ML 30 was induced by growth overnight in 0.5 mM IPTG.

medium. The accumulation of β-galactosides inhibited the uptake of P_i into aerobic cells (Table I). The inhibition varied from 50 to 70% between experiments. One-half of the inhibition was due to a diminished uptake of exogenous P_i into the endogenous pool of P_i within the cells, and the other half was apparently an inhibition of the incorporation of endogenous P_i into cell nucleotides.

The best condition for observing the inhibition of uptake of exogenous P_i into nucleotides by β-galactosides was one of low energy flux. This condition was achieved in aerobic cells by incubating the cells in the absence of P_i and succinate, and assaying at 20 °C. The inhibition became more complete as the incubation was extended prior to the assay, and was accompanied by a decrease in the rate of uptake of P_i into nucleotides. Adding exogenous succinate during the incubation or increasing the assay temperature, increased the rate of P_i uptake into nucleotides and decreased the inhibition due to β-galactosides. The inhibition was independent of P_i concentration between 0.01 and 1.0 mM, and was seen when $^{31}P_i$ was present before the addition of $^{32}P_i$ (both $^{31}P_i$ and $^{32}P_i$ were present at a final concentration of 0.01 mM).

IPTG, TDG, TMG and PTG were all active in inhibiting the uptake of exogenous P_i into cell nucleotides. Decreasing the concentration of TDG from 5.0 to 0.01 mM caused only a 30% decrease in the inhibition. A significant inhibition was not observed in ML 35 and in uninduced ML 30. Inhibition was observed in ML 30 with an induced *Lac* operon. Apparently, the uptake of exogenous P_i into cell nucleotides is inhibited by β-galactosides only in the presence of the product of the y gene,

that is, the M protein must have a β-galactoside bound to it in order to inhibit the uptake of exogenous P_i into nucleotides.

The inhibition of the uptake of exogenous P_i into nucleotides by TDG was demonstrable in spheroplasts and membrane preparations (Table II). However,

TABLE II

Inhibition by TDG of the uptake of P_i into nucleotides in various cell fractions. The assay procedure was the same as in Table I. The uptake data represent the incorporation of $^{32}P_i$ into nucleotides and $HClO_4$-insoluble material (see text for details). The uptake of $^{32}P_i$ is shown as nmoles incorporated P/mg protein per 15 min, and was measured in the absence of TDG. The final concentration of TDG was 0.5 mM and P_i was 0.01 mM. The values shown are the averages of duplicate determinations. Individual values ranged between ±10% of the mean.

Preparation	*Nucleotide**	*mg Protein/ ml incubation medium*	*P_i uptake*	*% Inhibition*
Cells	—	0.157	4.21	61
Spheroplasts	—	1.48	5.50	61
Spheroplasts	1 mM AMP	1.48	9.10	54
Spheroplasts membranes	—	1.13	7.20	55
Spheroplasts membranes	1 mM AMP	1.32	7.82	64
Spheroplasts membranes sonicate	1 mM AMP	0.58	0	0
Spheroplasts membranes	1 mM ADP	1.32	5.60	48
Spheroplasts membranes sonicate	1 mM ADP	0.58	8.80	−1
3000–40 000 × *g* pellet	1 mM ADP	0.19	5.00	−2
40000 × *g* supernatant	1 mM ADP	1.31	10.3	+2

* Nucleotides were present during the entire incubation at the concentrations shown.

the inhibition was completely lost upon sonication while the rate of P_i uptake into nucleotides increased. The incorporation of P_i into nucleotides in the cell-free fractions was dependent on ADP. In the 40000 × *g* supernatant, incorporation was inhibited 50% by 10 μM CCCP, a potent inhibitor of oxidative phosphorylation. Examination of the ability of the 3000–40000 × *g* pellet and the 40000 × *g* supernatant to incorporate P_i into nucleotides in the presence of 1 mM ADP, showed that about 93% of the total activity was in the 40000 × *g* supernatant.

Attempts at reconstituting the inhibition of P_i uptake in a cell-free system have been unsuccessful. Lyophilization, freezing, and storage at 0 °C caused reductions in the rate of P_i incorporation and destroyed the inhibition by β-galactosides. Solubilization of membranes with sodium dodecyl sulfate or Triton X-100 destroyed their ability to incorporate P_i into nucleotides.

There are two plausible interpretations for the inhibition of uptake of endogenous P_i into nucleotides. β-Galactoside accumulation might compete with the phosphorylation of nucleotides, for an intermediate of oxidative phosphorylation, resulting in a decrease in the rate of uptake of P_i into nucleotides. Alternatively, a nucleotide might be utilized for β-galactoside accumulation, producing an apparent inhibition of P_i uptake into nucleotides by lowering the nucleotide pool. To de-

termine which of these interpretations was correct, the turnover and level of the nucleotide pools were measured using two radioactive isotopes of phosphorus.

The effect of β-galactosides on the turnover and level of individual nucleotides

Figs 1 and 2 show the effect of TDG on the specific uptake (pulse label/total label, *i.e.* $^{33}P/^{32}P$) of P_i into aerobic and anaerobic cells and their nucleotides. The uptake of $^{33}P_i$ into cells was inhibited by TDG, and this inhibition was seen under both aerobic and anaerobic conditions (Fig. 1). TDG did not alter the endogenous

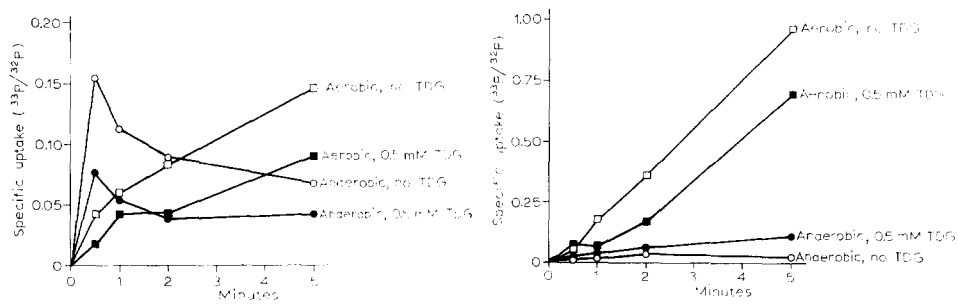

Fig. 1. Inhibition by TDG of P_i uptake into aerobic and anaerobic cells. Cells were grown in $^{32}P_i$ to achieve total labeling of cellular phosphorus. Starved cells were incubated with a final concentration of 50 μM $^{33}P_i$ and 0.0 or 0.5 mM TDG. At the times indicated cells were separated from the incubation mixture by membrane filtration, washed, and the activity of ^{32}P and ^{33}P was determined. This, and subsequent figures are representative data from one of two experiments.

Fig. 2. The effect of TDG on the P_i uptake into nucleotides of aerobic and anaerobic cells. Same experiment as in Fig. 1. Aliquots of cells were pipetted into ice cold $HClO_4$, centrifuged, and the ^{32}P and ^{33}P activity in the supernatant was determined. The specific uptake was corrected for the inhibition of P_i uptake into cells and changes in the pool of endogenous P_i.

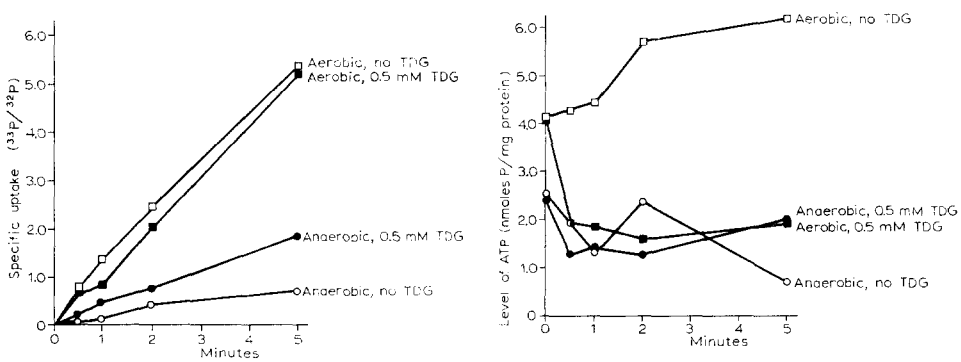

Fig. 3. The effect of TDG on the P_i uptake into ATP of aerobic and anaerobic cells. Same experiment as Figs 1 and 2. ATP was isolated by adsorption and deadsorption from charcoal, and polyethyleneimine–cellulose chromatography. The specific activity of ATP was determined by measuring ^{33}P and ^{32}P activity in the isolated ATP.

Fig. 4. The effect of TDG on the level of ATP in aerobic and anaerobic cells. Same experiment as Figs 1–3. The activity of ^{32}P per mg of cell protein was measured in the ATP fraction. The 50% drop in the ATP pool by TDG at 30 s in aerobic cells was exactly reproduced in a second experiment.

$^{32}P_i$ (20 nmoles P_i/mg cell protein) in aerobic cells, but caused it to rise from 20 to 40 nmoles P_i/mg cell protein during the 5-min incubation in anaerobic cells. The specific incorporation of P_i into nucleotides was slightly inhibited by TDG in aerobic cells, but markedly stimulated in anaerobic cells (Fig. 2); the same effect was seen in the specific incorporation of P_i into ATP (Fig. 3). TDG caused a small decrease (10 to 15%) in the total nucleotide pool of aerobic and anaerobic cells.

In aerobic cells, this decrease occurred within 30 s and could be largely accounted for by a lowering of the ribonucleoside triphosphate pools. The changes in ATP level are shown in Fig. 4; the other ribonucleoside triphosphates showed the same percentage change at 30 s. The level of ADP was not altered by TDG, while AMP increased to account for the drop in ATP. Since the accumulation of TDG did not change the specific activity of ATP and did not alter the level of ADP, but did lower the level of ATP, one can conclude that the accumulation of TDG inhibited the synthesis of ATP. This inhibition is more clearly seen if one plots $^{33}P_i$ uptake into ATP/mg cell protein (Fig. 5).

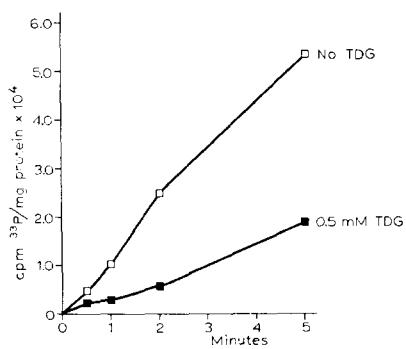

Fig. 5. The effect of TDG on the rate of ATP synthesis in aerobic cells. Same experiment as Figs 1–4. The data were calculated from Figs 3 and 4.

Figs 3 and 4 also show the effect of TDG on the turnover and level of ATP in anaerobic cells. The level was not affected by TDG, while there was a definite stimulation in turnover. A similar response to TDG was seen in the level and turnover of each of the other ribonucleoside triphosphates.

DISCUSSION

The observation that β-galactosides have the greatest inhibitory effect on P_i uptake and oxidative phosphorylation in aerobic cells with a low energy flux, suggests that β-galactosides are competing with ATP synthesis and with P_i uptake for a high energy intermediate of oxidative phosphorylation. The inhibition of P_i uptake was also seen in anaerobic cells, but ATP synthesis was stimulated. This result is consistent with the interpretation of the aerobic results, since in anaerobic cells ATP is generated by glycolysis and may be generating an intermediate of oxidative phosphorylation for β-galactoside accumulation and P_i uptake.

Fig. 6 shows a model for the transport of β-galactosides and the coupling of this transport to metabolic energy for accumulation. This model is similar to one

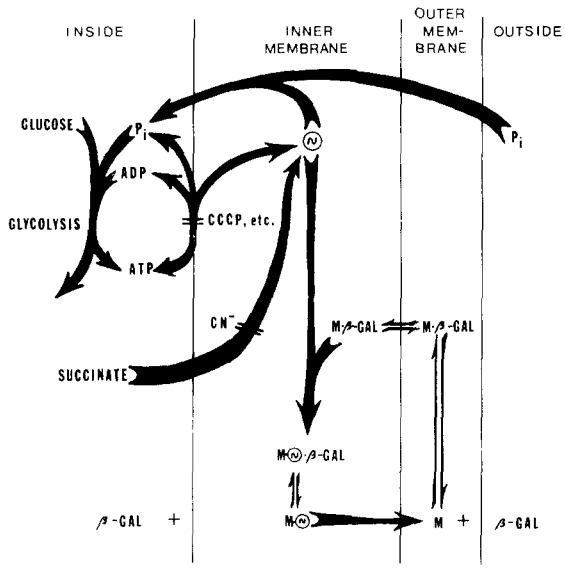

Fig. 6. Model of the coupling of metabolic energy to the β-galactoside transport protein (M protein) for the accumulation of β-galactosides. (~) represents a hypothetical high energy intermediate of oxidative phosphorylation. M-(~) represents a high K_m form of the β-galactoside transport protein which results from an interaction of M-β-galactoside with (~).

originally proposed by Fox and Kennedy[1], and to one proposed by Lardy *et al.* for cation accumulation in mitochondria[16]. The M protein, with its bound β-galactoside, is thought to react with an intermediate of oxidative phosphorylation to form an altered carrier in the inner part of the plasma membrane. The inner part of the plasma membrane is defined as that part which is accessible to a postulated high energy intermediate of oxidative phosphorylation (~). The altered carrier has a much lower affinity for β-galactosides. The high affinity form of the carrier is regenerated in the outer part of the plasma membrane. The inhibition by β-galactosides of P_i uptake into cells is pictured in this model to be a competition for the same intermediate of oxidative phosphorylation, although some other form of competition is possible. The nature of the intermediate of oxidative phosphorylation and its coupling site to the M protein remain uncharacterized. Recent reports by Kaback[17] suggest that the intermediate is generated aerobically between membrane-bound dehydrogenases and cytochrome b_1.

A mutant of ML 308 has been isolated which binds and transports β-galactosides as well as the parent strain, but accumulates them poorly[18]. As these authors suggest, this mutant may well be at the site of the energy link. If such a mutant maped to the y gene of the *Lac* operon, it would indicate that the M protein interacts directly with metabolic energy as our model suggests.

Our inability to measure the inhibition of P_i uptake *in vitro* may be a result of the fact that the M protein and the intermediate of oxidative phosphorylation interact in the hydrophobic environment of the plasma membrane, and any condition which disrupts the structure of the membrane such as detergents or sonication, would destroy the energy link.

From their observation that inhibitors of oxidative phosphorylation inhibited β-galactoside accumulation in anaerobic cells without affecting the level of ATP, Pavlasova and Harold[6] have postulated that oxidative phosphorylation provides the energy for β-galactoside accumulation. Since the inhibitors of oxidative phosphorylation also destroyed the H^+ gradient across the membrane, these authors suggested that a H^+ gradient was an essential condition for the accumulation of β-galactosides. This concept has been supported by the finding that protons are released by *E. coli* cells as lactose is accumulated[19]. Our experiments do not test this hypothesis. So little is known about the molecular events of oxidative phosphorylation, that the nature of the intermediate coupled to β-galactoside accumulation and how it is generated will have to await further characterization.

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REFERENCES

1 Fox, C. F. and Kennedy, E. P. (1965) *Proc. Natl. Acad. Sci. U.S.* 54, 891–899
2 Cohen, G. N. and Rickenberg, H. W. (1955) *C. R. Acad. Sci.* 240, 466–468
3 Kepes, A. (1960) *Biochim. Biophys. Acta* 40, 70–84
4 Koch, A. L. (1964) *Biochim. Biophys. Acta* 79, 177–200
5 Fields, K. L. and Luria, S. E. (1969) *J. Bacteriol.* 97, 57–63
6 Pavlasova, E. and Harold, F. M. (1968) *J. Bacteriol.* 98, 198–204
7 Cohen, G. N. and Rickenberg, H. W. (1956) *Ann. Inst. Pasteur* 91, 693–720
8 Buhler, D. R. (1962) *Anal. Biochem.* 4, 413–417
9 Kaback, H. R. and Stadtman, E. R. (1966) *Proc. Natl. Acad. Sci. U.S.* 55, 920–927
10 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
11 Josse, J. and Moffatt, J. G. (1965) *Biochemistry* 4, 2825–2831
12 Randerath, E. and Randerath, K. (1964) *J. Chromatogr.* 16, 126–129
13 Neuhard, J., Randerath, E. and Randerath, K. (1965) *Anal. Biochem.* 12, 211–222
14 Patterson, M. S. and Greene, R. C. (1965) *Anal. Chem.* 37, 854–857
15 Ernster, L., Zetterstrom, R. and Lindberg, O. (1950) *Acta Chem. Scand.* 4, 942–947
16 Lardy, H. A., Graven, S. N. and Estrada, O. (1967) *Fed. Proc.* 26, 1355–1360
17 Kaback, H. R. (1972) *Biochim. Biophys. Acta* 265, 367–416
18 Wong, P. T. S., Kashket, E. R., and Wilson, T. H. (1970) *Proc. Natl. Acad. Sci. U.S.* 65, 63–69
19 West, L. C. (1970) *Biochem. Biophys. Res. Commun.* 41, 655–661